Docket No.: 0259-0411PUS1
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Maurizio Dalle CARBONARE et al.

Application No.: 10/019,387 | Confirmation No.: 6340

Filed: March 26, 2003 | Art Unit: 1612

For: USE OF HYALURONIC ACID DERIVATIVES FOR THE PREPARATION OF PHARMACEUTICAL COMPOSITIONS AND BIOMATERIALS FOR THE PREVENTION OF THE FORMATION AND URE OF CUTANEOUS SCARS | Examiner: S. Maewall

DECLARATION UNDER 37 CFR 1.312

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Anna Maria Zanellato, do hereby declare the following:

1. Attached is a copy of my *curriculum vitae*.

2. I am working as Scientific Assistant to the Patent Department at Fidia Farmaceutica and I have worked in the field of cellular biology for 13 years.

3. I am familiar with the above referenced patent application, as well as the development, usages and properties of hyaluronic acid derivatives and their uses, in particular to reducing normotrophic scarring.

4. I have read and understand the subject matter of the Office Action of September 04, 2008.

5. The following experimental reports and comments are offered in support of the patentability of the instant invention.

6. I have attached as Attachments 1 and 2, the results of two studies conducted to evaluate the effect of different Hyaluronic Acid formulations on wound healing, particularly to compare results obtained by use of products described in the Davidson et al publication with results obtained by the present invention.

7. ***Experimental Report of Attachment 1*** - The report of Attachment 1 describes the test procedures and results for experiments conducted to evaluate the effect of HA formulations on increasing the area of repair in an animal wound model. The results of the experiment showed that us of the formulation according to the present invention (the HA benzyl ester formulation D and the auto-crosslinked formulation E) showed a marked and significant increase in wound coverage as compared to control and as compared to the formulation described by Davidson et al (the ethyl ester formulation C).

8. ***Experimental Report of Attachment 2*** - The report of Attachment 2 describes the test procedures and results for experiments conducted to evaluate the effect of HA formulations on cutaneous scarring. Treatment with the benzyl ester or the auto-crosslinked ester product resulted in unexpectedly reduced scarring as compared to treatment with either the ethyl ester as described in *Davidson* or with hyaluronic acid. The results are particularly summarized in the graph on of Attachment 2, from which it can be seen that as early as day 14 the scarred areas of the treatment groups (i.e. those treated with the auto-crosslinked or the benzyl ester according to the present invention) were <u>40% less extensive</u> than the control untreated areas; whereas, the wounds treated with either hyalastine (i.e. hyaluronic acid) or with the ethyl ester (as in Davidson et al) were <u>more</u> extensive than the control untreated areas. This means that scarring in the groups treated with either hyalastine or with the ethyl esters was actually <u>increased</u> as compared to control untreated animals; whereas, scarring was reduced by about 40% for those

groups treated with either the benzyl ester or the auto-crosslinked ester derivative according to the present invention.

9. Contrary to the results reported above, I would have expected the test compositions of the present invention to have the same activity as the control compositions because I studied the **Campoccia** reference, which, at page 2106 (column right, lines 3-8 and lines 24-29 with figures 5-6), shows as cell adhesion onto Hyaff 7 (of Davidson reference), Hyaff 11 and Hyaff11p75 (total and partial HA esterification) film surfaces, **is very different**: cell adhesion is similar for Hyaff 7 and Hyaff 11p75, i.e. a very low number of cell fibroblasts adheres to these films vs Hyaff 11 with total esterification, because Hyaff 11p75 may offer only a reduced number of surface points where cell proteins can adsorb and cell are able to adhere (page 2107). Cell fibroblasts are the most common cells of connective tissue and **play a critical role in wound healing**. It is evident to the person skilled in this art that, if this cell type does not adhere to surface film made by Hyaff 7 and Hyaff 11p75, these two esters **can not be used to enhance the healing process** because this process will be delayed. Therefore, the scarring process will be seriously compromised.

10. In addition, I would underline that Hyaff 11p75 causes the TNF production by cell seeded onto its surface, like LPS (pro-inflammation agent) (see figure 10); and, finally, films of Hyaff 11p75 cause significant effect on complement activation similar to those of Hyaff 7 because said films were found to be capable of causing the final production of the lytic complex (see page 2111, column left, lines 14-18 and 24-26, with figures 14 and 16). The complement activation is the sequential activation of serum components, initiated by an erythrocyte-antibody complex, producing an inflammatory response.

11. Therefore, studying all these references, I would have concluded that there are many problematic aspects regarding the use of Hyaff 7 and the very similar Hyaff 11p75 for the healing process and, as a consequence, for the related scarring process. But, on the contrary, I unexpectedly found that the test compositions (the HA benzyl ester and auto-crosslinked

derivative) are **able to prevent the formation of scarring better than a single application of control composition.**

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated:
02/03/2009

By _____
Anna Maria Zanellato

ATTACHMENT 1

Hyaluronate Derivatives and their application to Wound Healing models (Davidson Reference)

New experiments have been conducted to evaluate the effect of different HA formulations in increasing the area of repair in a wound animal model

Materials and methods

Wound model:

- split-excisional wound in the pig

Tested formulations:

A. **alginate vehicle** (3% NA alginate, pH 7 in saline)
B. 0.2 % **hyaluronic acid** (Connettivina ®) in alginate vehicle)
C. 0.2 % **hyaluronic acid ethyl ester** (Hyaff 7 p75) suspended in alginate vehicle
D. 0.2% **hyaluronic acid benzyl ester** (Hyaff 11 p75) suspended in alginate vehicle
E. 0.2% **autocrosslinked hyaluronic acid** (ACP) (with a degree of 5% of the carboxyl groups crosslinked) suspended in alginate vehicle
F. **occlusive dressing** (Op-Site™ - semipermeable polyurethane dressing)

Experimental Procedure

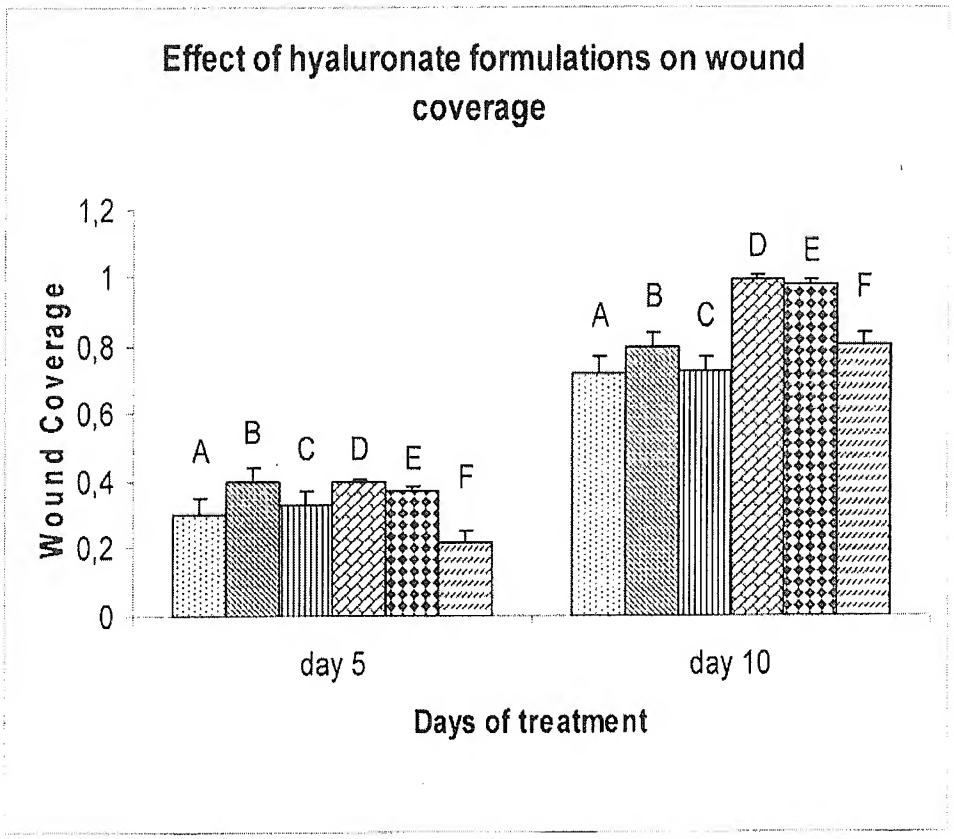
All treatments were performed under general anaesthesia using intramuscular injections of ketamine/xylazine, following standard protocols.
Three 40-kg male, 2-3 months old, outbred pigs, obtained from a commercial breeder, two days prior surgery received an intramuscular injection of 40 mg of methylprednisolone to retard the physiological healing process. For excisional wounds, a standardized 2x2 cm full-thickness wound of 1.2-1.4 mm depth was made; each animal received 6 wounds along each flank, arranged randomly **for daily treatment** with the formulations to be tested. All wounds, including those receiving no treatment, were covered with Op-Site™ to retard dehydratation of the wound site, to retain the formulations *in situ* and to avoid or prevent contamination.

Analysis of the wound healing included morphometric parameters of repair. Overlying regenerated epidermis were easily teased away and weighed.
Epithelial coverage was quantified by computerized morphometric analysis of tissue sections. **Re-epithelialization was scored as the total, integrated length of epidermal coverage** (from both wound margins and epidermal appendages).

Results

Excisional wounds were evaluated at **5** and **10** days after surgery. On day **5**, all of the gel formulations containing HA showed a trend towards increased epidermal coverage, with slight differences among them; by day **10**, when wound coverage had reached nearly 80%, results with formulations B and C were not significantly different from control values, while formulations **D** and **E** demonstrate a **marked and significant increase** of the wound coverage values vs all control samples and vs hyaluronic acid ethyl ester (Graph 1).

Graph I



A. **alginate vehicle** (3% NA alginate, pH 7 in saline)
B. 0.2% **hyaluronic acid** (Connettivina ® in alginate vehicle)
C. 0.2% hyaluronic acid ethyl ester (**Hyaff 7 p75**) suspended in alginate vehicle
D. 0.2% hyaluronic acid benzyl ester (**Hyaff 11 p75**) suspended in alginate vehicle
E. 0.2% autocrosslinked hyaluronic acid (**ACP**) (with a degree of 5% of the carboxyl groups crosslinked) suspended in alginate vehicle
F. **occlusive dressing** (Op-Site™ - semipermeable polyurethane dressing)

ATTACHMENT 2

Decrease in the area of cutaneous scarring in a rat model following treatment of the wound with the Hyaluronate derivatives and Hyaluronic acid

The animals (n= 32) were sedated by intramuscular injection of ketamine/xilazine (0,1 mg/g).
The backs of the animals were shaved, washed and disinfected with chlorhexidine and iodate solution.
Four full-thickness wounds were performed on each animal using a punch with a 6 mm diameter.

Treatment of wounds:

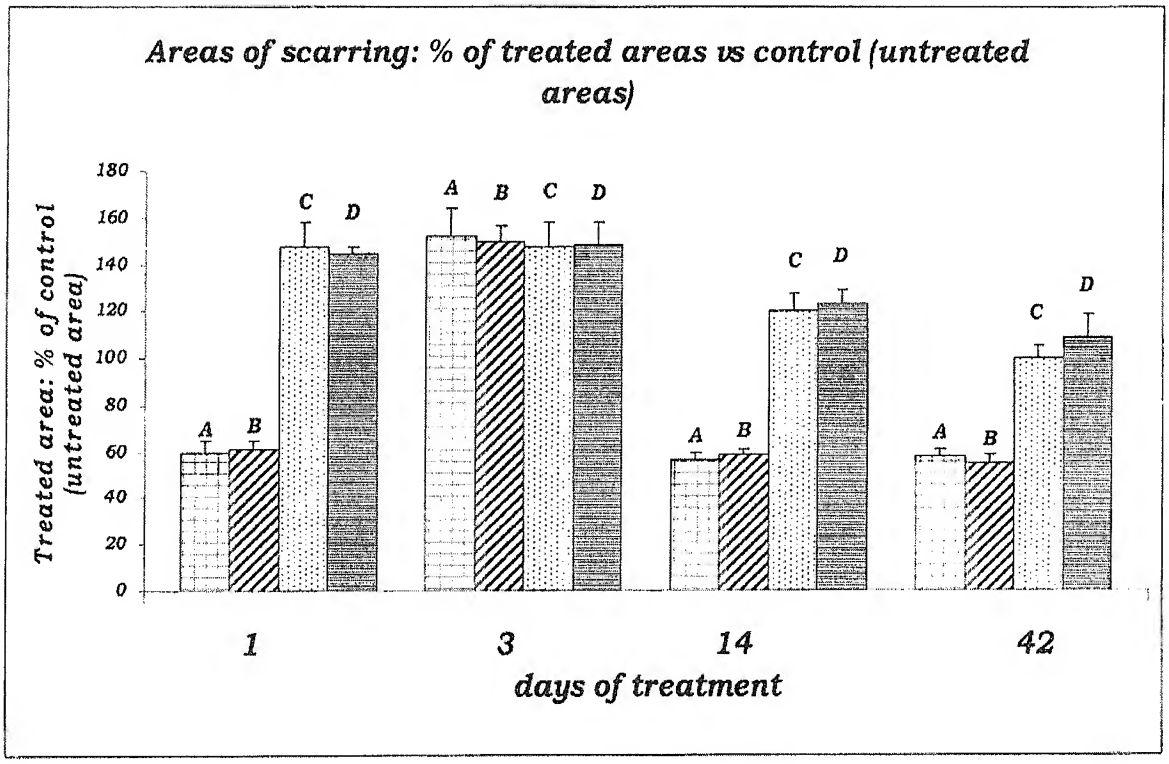
Groups	Number of treated sites	Treatment
A	16	Partial benzyl ester of hyaluronic acid **Hyaff 11p75** in the form of non-woven fabric
B	16	Auto-crosslinked ester of hyaluronic acid in the form of gel **(ACP)** 60 mg/ml
C	16	Partial ethyl ester of hyaluronic acid **Hyaff 7p75** in the in the form of gel 60 mg/ml
D	16	**Hyaluronic acid**, Hyalastine® fraction, 60mg/ml

Tested formulations:

1. Partial benzyl ester of hyaluronic acid **Hyaff 11p75** in the form of non-woven fabric (US 4,851,521-EP216453)
2. Auto-crosslinked ester of hyaluronic acid **(ACP)** (US 5,676,964-EP 341745) with a degree of 5% of the carboxyl groups crosslinked by ester bonding to hydroxyl groups to the same hyaluronic acid molecule and/or to hydroxyl groups of different hyaluronic acid molecules.
3. Partial ethyl ester of hyaluronic acid **Hyaff 7p75** in the in the form of gel (US 4,851,521- EP216453)
4. **Hyaluronic acid**, Hyalastine® fraction (US 5,925,626-EP 138572)

Two wounds in each animal were treated **by a single application** of tested formulation, and **two were used as control, i.e. untreated sites.**
4 treated areas for group were removed at set times (1, 3, 14, 42 days). The samples were cut into sections and stained with Mallory's triple stain; the sections were analysed by optical microscope and the **scarred areas were measured.**
The graph reports values expressed as percentages of scar area of the treated sites compared to that of the untreated sites, and each value corresponds to the mean of four determinations on two different animals.

It is evident that a single application of partial benzyl ester of hyaluronic acid, **Hyaff 11p75,** and of the auto-crosslinked ester of hyaluronic acid, **ACP**, is able to prevent the formation of scarring better than a single application of hyaluronic acid and of partial ethyl ester of hyaluronic acid, **Hyaff 7p75** .
Indeed, as early as the 14th day, it is possible to observe that the scarred areas of the group treated with an **auto-crosslinked ester** of hyaluronic acid and with partial **benzyl ester** of hyaluronic acid, are **40 %** less extensive than the control untreated areas, while in the case of the sites treated with Hyalastine® and with **Hyaff 7p75,** the scarred areas are more extensive than the control untreated areas.



A Partial benzyl ester of hyaluronic acid, **Hyaff 11p75**, in the form of non-woven fabric
B Auto-crosslinked ester of hyaluronic acid in the form of gel, **ACP**, (60 mg/ml)
C Partial ethyl ester of hyaluronic acid, **Hyaff 7p75**, in the in the form of gel (60 mg/ml)
D **Hyaluronic acid**, Hyalastine® fraction, (60mg/ml)